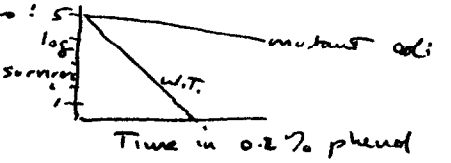
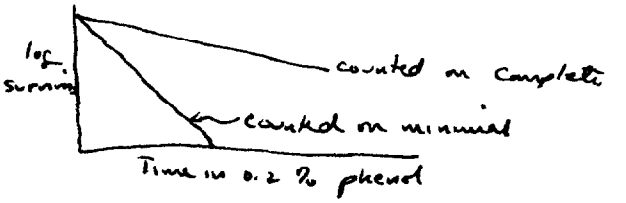
Oct 25, 1957

Dear Josh,

At the time Ryan left, the phenol business looked wonderful - we got reconstruction results like this:



But then we discovered that the differential was due the fact that we happened to be counting mutant survivors on complete agar, and wild-type on minimal. There, wild type E. coli given the following:



It seems a "phenol-killed" bug is dead only insofar as colony-formation on minimal is concerned - something in complete agar "reactivates" them. (There is a small % of the population that is irreversibly killed)

On rechecking mutants for killing in phenol-minimal with and without growth-factor, we found just the reverse of what Hobby, Meyer & Chaffee state (they claimed phenol acted like penicillin.) In our hands, an E. coli mutant survived better with its growth factor than without.

We thought the difference might be due to ~~Ryan~~ their using Gt organisms, so we tried a B. subtilis mutant. The first run, we got no differential at 0.2%, growth in 0.1%, but the desired differential (better survival without growth-factor) at 0.15%! Then we couldn't repeat it. We have dropped it there, tho' I feel that there may be something we're missing. You're welcome to take over if you can figure out what's wrong.

We just heard, with much excitement, about the Bacteriological velvet," and are converting our factory here to your rubber-stamp method immediately. Did you have to have rings made, or are ready-made ones available?

We have just worked out something of which we are

quite pound, and in Neuro I'm sure you'll be interested. We are using penicillin to select for auxotrophs without scattering the clones arising during intermediate cultivation; i.e., we get each original mutation as a single colony. Here's how:

1) Grow wild-type E. coli (9637) in minimal, dilute and irradiate in minimal killing from $10^8 \rightarrow 10^7$/ml. (Use log-phase cells)
2) Immediately dilute in min, and plate out in minimal between protective layers, at about 2×10^3 survivors per plate.
3) Incubate at 32° for 7-8 hours. (Each wild-type survivor becomes a micro-colony of about 100 cells, while each mutant (roughly 50 per plate) divides until auxotrophy is expressed. Probably at least 10 cells are formed per mutant colony.
4) We now layer with penicillin-minimal solution, and incubate 24 hrs.
5) Layer with penicillinase solution (Schenley, 1 unit/100 units penicillin, or about 100 units/plate). Place at 5°C for 12 hours to allow diffusion.
6) Incubate at 37° for 48 hours. Mark wild-type colonies (we get about 30-40/plate.)
7) Layer with complete agar. 24 hours later mutant colonies are up at a perfectly reproducible 2 to 3 % of survivors of irradiation, i.e., about 50/plate if you plate 2×10^3 U.V.-survivors. Wild-types are completely absent from the 48-72 hour crop.

We have picked and tested, and are getting a variety of types. The nice thing is that each colony is an original mutation.

If you don't layer with complete, there is a slow development of mutant colonies anyway, due to cross-feeding or leaking from pen-killed cells. But if you provide with a single growth factor, you could pick fairly efficiently. We are working on minimizing the number of "undesired" mutants that come up in minimal, with the goal of having to pick only a desired class of mutants.

Will be publishing shortly, I hope, and will be interested to hear the results in case you decide to try it. What is your impression?

Please send your 1951 reprints, and keep up the wonderful work - hope to hear from you soon,

Best to Esther,

Ed